The Need for Better Methods of Extrapolation

by Leo Friedman*

In the fall of 1966 the Food and Drug Administration established an Advisory Committee on Protocols for Safety Evaluation. The first problem to be reviewed was the requirement for reproduction studies in the safety evaluation of pesticides and food additives, and the adequacy of the current methods involved. Reproduction studies are designed to give information on: (1) effects on male and female fertility, (2) effects during gestation on the mother and the fetus, (3) effects appearing after parturition on the mother (for example, lactation) and the offspring (for example, growth, development, sexual maturation), and (4) mutagenic effects, particularly those which might not appear in the first generation.

The report of panel dealing with this problem was written in November 1968 and published later (*1*). During the time the panel was considering the problem, a report prepared by the Genetic Study Section under the chairmanship of Dr. James F. Crow (*2*), became available to us (C. C. Cockerham, personal communication). The emphasis in this particular report of the Genetic Study Section was that there is reason to be concerned about chemicals as a mutagenic risk equivalent to radiation, possibly even more serious. The concern, of course, is with the welfare of future generations as well as with the health of contemporary populations. It is useful to emphasize again that methods to detect mutagenic effects that are useful for the evaluation of mutagenic risks must deal with the potential for genetic changes that are transmissible to future generations. Without this clearly in mind we can easily become confused regarding the import of our laboratory findings.

I believe it is of interest to this group to cite certain portions of the Reproduction Panel Report (*1*).

1. "There should be a repository of information on the mutagenic potential of chemicals. Such information should include chemical nature, quantitative information on mutagenicity, the particular test systems on which this information is based, and other biological effects including carcinogenesis."

2. "The task of demonstrating that a chemical constitutes no genetic hazard is somewhat different and inherently much more difficult than carrying out and evaluating other toxicity tests including reproduction tests. The 'permissible level' takes on new meaning when multiplied over future generations. Mutagens often act at very low concentration. Definitive tests of their effects in mammals are tedious and expensive. Generally, the more sensitive and feasible the screening test is for chemical mutagens, the further the test organism is removed from man. Little information is available about the applicability of non-mammalian assay systems to mammals."

3. "Because there is already evidence that some chemicals are mutagenic in some test systems, all new chemicals to which human

*Division of Toxicology, Food and Drug Administration, Washington, D. C. 20204.

populations are to be exposed should be tested in some way for mutagenic effects. The most definitive tests (specific locus tests and backcross or genetic load tests) for chemical mutagenesis in mammals are clearly impractical because they require approximately one-half million animals, or more, depending on test circumstances, to detect a doubling of the mutation rate. Feasible is the dominant lethal test in mice and/or rats as outlined by Bateman" (*3*).

"It is likely that occasionally a chemical, which does not constitute a hazard to human health under the actual conditions of use, might be shown to be mutagenic by simpler test systems. For example, a chemical when added to the culture media of microorganisms might produce a detectable increase in mutation frequency, but be innocuous to man because of degradation in the gut, detoxification, or other reasons. The same comments are appropriate for cytological tests with cultivated cells. Further tests would be in order for a chemical which is suspect as might be indicated by positive results of simple screening tests or by existing information such as known mutagenicity of related compounds already recorded in the registry."

"Test procedures in simple systems are also open to the criticism that negative results do not prove that a chemical will not be mutagenic to man. Among possible reasons for this are the high degree of specificity of the tests and production of active metabolites. Consequently, the proper evaluation of the non-mutagenicity of chemicals may be accomplished best through a series of tests, first being direct tests on microorganisms for specific effects and on cultivated mammalian cells for chromosomal aberrations. In the event that results of direct tests are negative (which still leaves the possibility of mutagenic metabolites being formed in the animal) or that the potential value of the substance warrants the effort, tests in mammalian systems should be undertaken.

4. "The simple bacterial tests are for back mutations, and only if mutagens produce both forward and backward mutations with roughly equal frequencies are these tests adequate. Since results of different systems do not always agree, at least two bacterial systems should be used to test for specific effects."

"Of the tests currently available (November 1968) using mammalian systems, three types are suggested: (1) dominant lethal tests in mice (possibly rats); (2) cytological tests of cells removed from treated animals, and (3) mammalian host-mediated microbial assays. If results of a dominant lethal test and of one other test in a mammalian system as well as results of the 'direct tests' are negative, the chemical may be judged to be nonmutagenic. When the results of 'direct tests' are positive, additional data comparing the metabolism of the compound in the animals used in the mammalian tests to its metabolism in man should be available before the hazard of mutagenesis in humans can be evaluated."

Since this report was written there has been very rapid development in mutagenesis assay systems and their application to a great variety of substances. The specific concern over the contributions of environmental agents to the problem of genetic disease was thus widely recognized and we are now at an important crossroad as we recognize the need to better equip ourselves for the task of extrapolating from the results of our laboratory studies to the potential risk to human populations.

The Mrak Commission Report (*4*) contained a chapter on mutagenicity of pesticides. The various methods for mutagenicity testing were fully reviewed by the Mutagenicity Panel and a recommended program for mutagenicity testing was set forth. The recommended protocol included the test of each compound in three mammalian systems, the dominant-lethal, host-mediated, and *in vivo* cytogenetic, by appropriate methods of administration reflecting human exposure and also parenterally and at high dose levels such as maximal tolerated doses. Also, each compound should be tested in ancillary microbial systems, preferably those detecting both single nucleotide changes and effects involving more than one gene.

The precision of testing such systems would be such that doubling of the control level of mutation would be statistically significent at the 5% level. If one or more of the three mammalian tests shows a significant effect, the test is regarded as positive. A pesticide is regarded as negative if none of the tests is significantly different from its control. If only the microbial test is positive, more detailed mammalian tests are indicated.

These recommendations and others not quoted indicated, on the one hand, a degree of confidence in the precision and interpretability of results from these methods, and on the other, a degree of uncertainty which accurately reflected the lack of experience of all concerned with the problems of methodology and with the interpretation of results from mutagenesis testing.

There seems to be agreement among two separate panels that the dominant lethal tests on rodents, somatic and/or germinal cell cytogenetics and the newly developed host-mediated assay were the methods of choice in any program of safety evaluation that was to include specific attention to the problem of mutagenesis. Therefore, when the Food and Drug Administration, in response to the directive of the White House Conference on Nutrition, undertook to re-examine the substances used in food that had been listed as generally recognized as safe (GRAS), it was logical that the question of mutagenesis should be seriously considered. All of these substances had had a considerable history of human use but had not been studied as extensively as any new food additive. There were gaps in knowledge, therefore, especially in regard to newer questions such as mutagenicity and teratogenicity, and these could be dealt with only by application of appropriate laboratory methods.

Therefore, about a year or two ago, the FDA undertook mutagenicity testing of GRAS compounds on a relatively large scale. From the beginning it was recognized that not only were substances being tested but that new laboratories which had contracted to undertake the task were acquiring experience in a new area, and that the test systems themselves were being scrutinized.

The Mrak Commission Panel had stated that "the testing procedures recommended above must be constantly updated and improved to reflect new techniques and new data." This large-scale undertaking by FDA would provide new experience and data, and would inevitably have an impact on our understanding of the methods used and our confidence in results and our ability to interpret these results in terms of the risk to human populations.

I believe we can all agree that essential to any procedure used for safety evaluation is an acceptable degree of reproducibility. Equally important is that the test procedure relate to a health hazard to which man is known to be susceptible. For instance, we know that people are poisoned in the same way that our laboratory animals are when we determine lethal doses. We know that man is subject to cardiovascular effects, carcinogenicity, and teratogenicity and other chronic and acute health problems; so that studies of these specific problems in animal models are easily translatable, at least conceptually.

The situation with mutagenicity testing has been very different than that where biological endpoints have been traditionally used. Here the endpoints are not always clearly related to the structural or functional effects which are of known concern to man. Indeed, the relationships seem sometimes on tangential and based on purely mechanistic consideration. It is assumed, for example, that if the compound is found to induce a sex-linked recessive lethal mutation, it would also induce an increased frequency of galactosemia, phenylketonuria, or any number of other recessive conditions in which the specific gene product has been rendered functionless by mutation. This concept is supported better by theory than by observation or direct demonstration, but it points out an essential fact. A great deal more is known about mutagenesis in terms of mechanism than is true for most areas of toxicology. In mutagenesis, the chemical nature of the

target substance is known and the molecular mechanisms are at least as well understood as any event in biology. Thus, mutagenesis may occupy a special place in toxicology wherein test system endpoints and human health effects may possibly not be as stringently related as appears necessary in other areas.

On the other hand, we must be certain that our experimental model is relevant to the problem, that we are comparing apples to apples, and not to oranges. We must, therefore, identify the genetic events which are of concern to man and focus our efforts on these events by selecting test systems which are suitable for such purposes.

Presumably, we know a good deal about the types of mutations which are of concern in man from the specific protein products generated by mutation. For instance, it is known from studies of the globin molecule that gene mutations in man include point mutations. These have either been shown or presumed to include base substitution or base addition or deletion. Small chromosomal deletions either of a terminal or interstitial nature are also known to be responsible for gene mutations in mammals and as such deserve our concern. While there are a number of systems capable of measuring any one of these effects, we need to select the one or ones most closely relevant to man. We further need to be mindful of the need to assess the effects and consequences of pharmacokinetic factors upon the compound in question. Ultimately, we must derive quantitative information in sufficiently precise terms to define mutagenic risk.

In addition to gene mutations, it is obvious that man is subject to a significant cytogenetic disease burden as exemplified by Down's, Klinefelter's and Turner's syndromes and a variety of other conditions known to be of cytogenetic origin. These heritable conditions are predicated on certain types of cytogenetic anomalies but it is not known whether these anomalies are induced by the same chemicals which are capable of inducing gene mutations and if so, whether such alterations can accumulate within the germinal stem line and be transmitted to future generations. Such information is necessary if we are to achieve proper understanding of the problem leading towards effective safety or risk evaluations. Indeed it is appropriate to cite Brent, who emphasized: Do not adopt a testing protocol whose results are uninterpretable for human situation (*5*). Clearly, we must address ourselves to this central question and objectively examine and evaluate all mutagenicity tests in terms of whether they can or will provide the kind of information which is applicable to human safety evaluation. This is the important charge for this Workshop.

Specifically, we must ask the following question of any current or proposed mutagenicity test system: (1) Are the events measured heritable and to what extent would they accumulate either in germinal stem cells or within the human population? (2) Does the assay properly account for all essential pharmacokinetic factors relevant to human exposures? (3) Are the genetic events measured of concern to human health? (4) Is the assay applicable to the types of exposures which are of greatest presumed hazard to man, i.e., what is the probability of a given dose reaching a given number of people? (5) To what extent can the assay yield quantitative information useful in ranking chemical mutagens in terms of mutagenic potency?

If this Workshop Study Group agrees that these questions are essential ones to ask, the suggestion is made that they serve as a basis for the panel discussions.

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